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Antioxidant activities of the extracts of the aerial roots of *Pothos aurea* (Linden ex Andre)

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ABSTRACT

The petroleum ether, ethyl acetate and chloroform extracts of the aerial roots of Pothos aurea intertwined over on Lawsonia inermis (MM) and Areca catechu (MP) were examined for their phytochemical constituents and for their in vitro activities using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay and reducing power assay. Higher antioxidant potential of the extracts was observed in both DPPH scavenging assay and reducing capacity assay.

Key words: *Pothos aurea*, Antioxidant properties, DPPH assay, Reducing power assay.

INTRODUCTION

Pothos aurea is a popular house plant with numerous cultivars. In tropical regions *Pothos* is grown as a ground cover or as a scrambler up trees. This evergreen root- climber has a slender twining and branching stem that grows up to 65 feet long. The glossy, heart-shaped, waxy leaves are bright green or irregularly splotched or marbled with yellow or cream. It is very efficient at removing indoor pollutant such as formaldehyde, xylene and benzene [1].

The aerial roots of *Pothos aurea* climbed over two different kinds of trees like, *Lawsonia inermis* (mehandi), *Areca catechu* (betal nut palm) has been taken for the studies because the assumption of different properties of the two host plants. The two plant samples are given the acronym MM and MP for *Pothos aurea* climbed on *Lawsonia inermis* and *Pothos aurea* climbed on *Areca catechu* respectively.

Antioxidants help organisms deal with oxidative stress, caused by free radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. The quest for natural antioxidants for dietary, cosmetic and pharmaceutical uses has become a major industrial and scientific research challenge over the last

two decades. Efforts to gain extensive knowledge regarding the power of antioxidants from plants and to tap their potential are therefore on the increase. Several methods are used to measure the antioxidant activity of a biological material. The most commonly used ones are those involving chromogen compounds of radical nature that stimulate the reductive oxygen species. These methods are popular due to their ease, speed and sensitivity. The presence of antioxidants leads to the disappearance of these radical chromogens; the most widely used ones being the DPPH methods.

In view of the aforesaid in the present study the antioxidant activity of petroleum ether, ethyl acetate, chloroform, extracts of MM and MP of *Pothos aurea* were assessed using DPPH (1, 1 diphenyl-2-picrylhydrazyl) radical quenching and reducing capacity assay.

MATERIALS AND METHODS

Collection of plant

Aerial roots of *Pothos aurea* intertwined over the *Lawsonia inermis* were collected from Coimbatore and that intertwined over *Areca catechu* was collected from Palakkad District. The selection of the roots from two different hosts was made on availability.

Preparation of extracts

Solvent extraction of the aerial roots of MM and MP (Petroleum ether, ethyl acetate and chloroform) were prepared by refluxing for 12 hours with the corresponding solvent.

Assessment of Antioxidant activity

DPPH assay: (1, 1-diphenyl-2-picrylhydrazyl)

Principle

The scavenging reaction between (DPPH.) and an antioxidant (H-A) can be written as:

$$\underset{\text{(Purple)}}{(DPPH) + (H\text{-}A)} \longrightarrow \underset{\text{(Yellow)}}{DPPH\text{-}H + (A)}$$

Antioxidants react with DPPH, which is a stable free radical and is reduced to the DPPHH and as consequence the absorbance's decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability [2].

Chemicals required:

1.0.1 Mm solution of DPPH in methanol was prepared and used in the study.
2. Ascorbic acid (1%)

Procedure

0.1 Mm solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentration. Thirty minutes later, the absorbance was measured at 517 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (1 to18 µg/ml) was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{DPPH Scavenged (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of the control reaction and a test is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the *Pothos aurea* extract is expressed comparing with standard ascorbic acid.

Reducing power assay

Principle

The reducing power of petroleum ether, ethyl acetate and chloroform extracts of MM and MP was determined by the method of Oyaizu [3]. Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

$$\text{Potassium ferricyanide} + \text{Ferric chloride} \xrightarrow{\text{Antioxidant}} \text{Potassium ferrocyanide} + \text{ferrous chloride}$$

Chemicals required

Potassium ferricyanide (1% w/v), phosphate buffer (0.2 M, pH 6.6), trichloro acetic acid (10%), ferric chloride (0.1%) and ascorbic acid (1%).

Phosphate buffer preparation

0.2M dibasic sodium phosphate (35.61g in 1 lit) and 0.2M monobasic sodium phosphate (31.21g in 1lit) was prepared. Mixed 18.75 ml of 0.2M dibasic sodium phosphate with 31.25 ml monobasic sodium phosphate and diluted to 100 ml with water.

Procedure

Various concentrations of the plant extracts in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50^0C for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min whenever necessary. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power.

$$\textbf{\% increase in Reducing Power} = \frac{A_{test}}{A_{blank}} - 1 \text{ X } 100$$

where A_{test} is absorbance of test solution; A_{blank} is absorbance of blank. The antioxidant activity of the *Pothos aurea* extracts were compared with the standard as acids.

RESULTS AND DISCUSSION

DPPH assay

The DPPH radical scavenging assay is an easy rapid and sensitive method for the antioxidant screening of plant extracts. A number of methods are available for the determination of free radical scavenging activity but the assay employing the stable 2, 2-diphenyl-1-picryl-hydrazyl radical (DPPH) has received the maximum attention owing to its ease of use and its convenience [4].

Table 1: DPPH scavenging activity of ascorbic acid

Concentration of ascorbic acid 1ml=3µg	% of increase DPPH Scavenging (ascorbic acid)
1ml	63.51
2ml	64.86
3ml	66.21
4ml	67.56
5ml	68.91

Table 2: DPPH scavenging activity *Pothos aurea* extracts

Extracts of MM and MP	% of increase DPPH scavenging (MM)	% of increase DPPH scavenging (MP)
Petroleum ether	67.56	64.86
Chloroform	59.45	58.10
Ethyl acetate	56.75	64.86

The results of the assay are expressed in percentage (%) of inhibition of DPPH free (**Table 1**).The DPPH assay of reference compound ascorbic acid given in **figure 1**. The analysis of (**Table 2**) that the radical scavenging activity of the MM extracts of *Pothos aurea* increases with increasing in concentration and follows the given orders **PE > CH > EA** where as MP extracts **PE> EA>CH (Figure 2).** The extracts of the aerial roots of *Pothos aurea* MP also showed good anti oxidant activity. Ethyl acetate extract MP showed higher activity compared to the other extracts. The aqueous extract of MP showed comparatively better activity (20%) than MM extract (14%). Compared to MM and MP extracts PE extracts shows a good activity than the other extracts.

Reducing Power Assay

Around 10-15mg of the extracts gave 68% DPPH scavenging activity. From the present results it may be postulated that the extracts of *Pothos aurea* reduces the DPPH radical to corresponding hydrazine when its reacts with hydrogen donors in antioxidant principles.

In this assay the yellow colour of the test solution changes to various shades of green and blue is depending upon the reducing power of each compound. The presence of radicals (ie antioxidant) causes the conversion of the Fe^{3+} / ferricyanide complex used in this method to the ferrous form. Therefore by measuring the formation of pearls Prussian blue at 700nm [5] we can monitor the Fe^{2+} concentration; a higher absorbance at 700nm indicates a higher reducing power. The results of the reducing power assay are given in **Table 3.**

Figure 1: DPPH scavenging activity of ascorbic acid

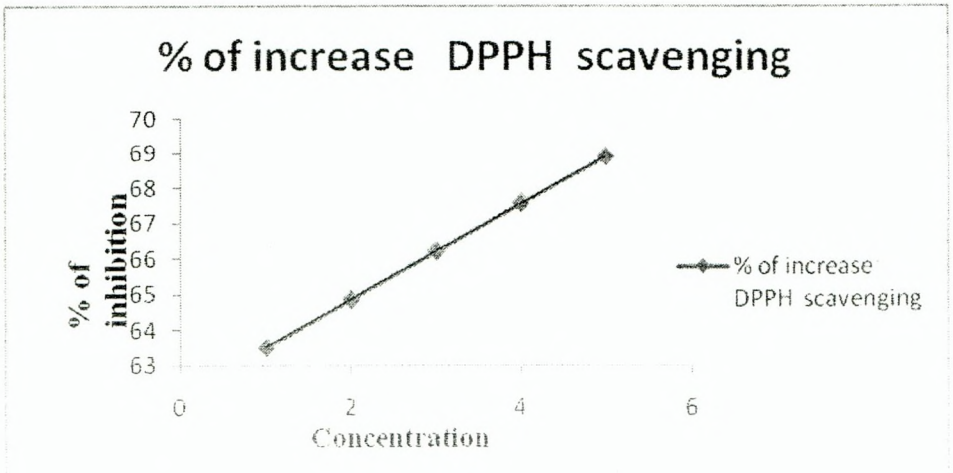
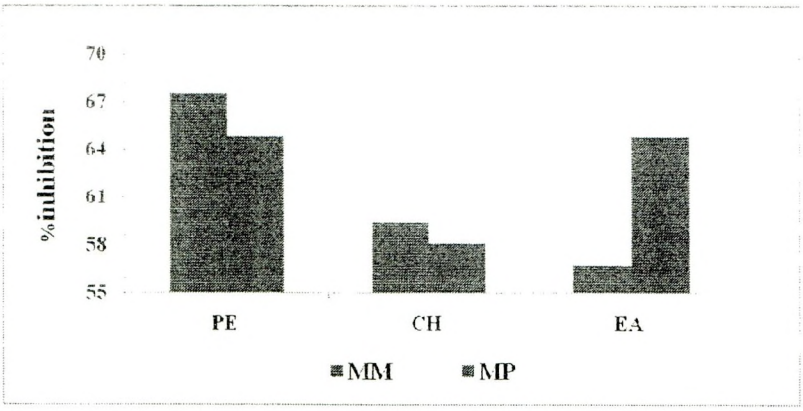


Figure 2: DPPH scavenging activity of *Pothos aurea* extracts



The reducing power assay of reference compound ascorbic acid given in **figure 3** the relative reducing power of the different solvent extracts of MM and MP of *Pothos aurea* **(Figure 4).**

Table 3: Reducing power capacity of ascorbic acid

Concentration of ascorbic acid 1ml=300µg	Absorbance (700nm)
2ml	0.22
3ml	0.32
4ml	0.45
5ml	0.55
6ml	0.65
7ml	0.75

Table 4: Reducing power capacity *Pothos aurea* extracts

Extracts of MM and MP	Absorbance (MM)	Absorbance (MP)
Petroleum ether	0.33	0.23
Chloroform	0.35	0.25
Ethyl acetate	0.37	0.26

Figure 3: Reducing power assay capacity of ascorbic acid

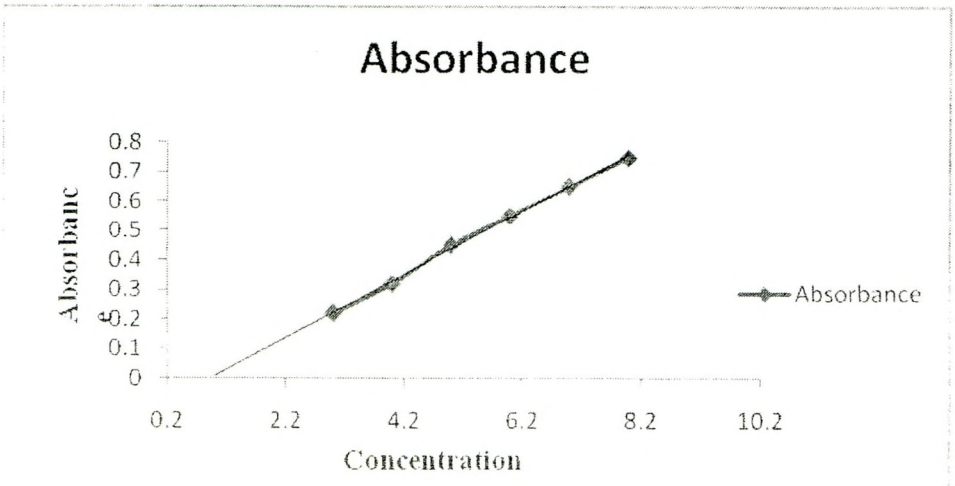
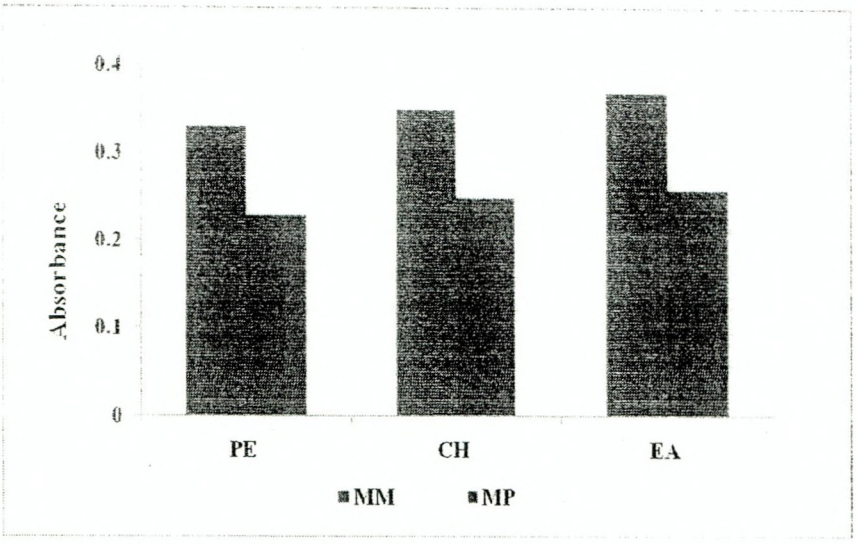


Figure 4: Reducing power assay capacity of *Pothos aurea* extracts



The higher absorbance of EA extracts may be due to its strong reducing power potential. Comparing MM and MP extracts, the MM extracts showed better reducing power than MP extracts. The reducing power of the extracts may be due to the biologically active compounds in the extract which posse's potent donating abilities. This assay further confirmed the antioxidant properties of the extracts.

CONCLUSION

In conclusion this work describes for the first time *invitro* antioxidant activity of the extracts of the fallen plant parts of *Pothos aurea.* Most of the extracts showed excellent free radical scavenging activity. Literature reports are evident that the reducing power of bioactive compounds is associated with antioxidant activity thus a relation is evidenced between reducing power and the antioxidant effect.

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